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Appl. No. 10/699,449
Amendment dated August 2, 2006
Reply to Office Action of April 3, 2006

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently Amended) A method comprising
 - a) obtaining a mixture comprising a glycosylated protein and unglycosylated proteins, wherein the glycosylated protein comprises a protein having a glycosylation site and a glycosyl group bound to the protein via the glycosylation site,
 - b) contacting the mixture with a resin, wherein the resin comprises a nucleophile bound to a solid support via a linker, said contacting done under conditions sufficient to remove the glycosyl group by β -elimination from the glycosylated protein to yield a deglycosylated protein having ~~a~~-an unsaturated intermediate at the deglycosylation site, the deglycosylated protein bound to the solid support via the unsaturated intermediate at the deglycosylation site;
 - c) rinsing the bound deglycosylated protein, thereby removing unglycosylated proteins;
 - d) releasing the deglycosylated protein from the solid support.
2. (Original) The method of claim 1 wherein the mixture comprises a plurality of glycosylated proteins.
3. (Original) The method of claim 1, further comprising dephosphorylating proteins of the mixture prior to contacting the mixture with the resin.
4. (Original) The method of claim 1, further comprising subjecting the released proteins to mass spectrometric analysis.
5. (Original) The method of claim 1, further comprising subjecting the released proteins to analysis by gel electrophoresis.

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6. (Original) The method of claim 1, further comprising subjecting the released proteins to analysis by HPLC.

7. (Original) The method of claim 1 wherein the nucleophile comprises a moiety selected from a thiol moiety, an amine moiety, and a hydroxyl moiety.

8. (Original) The method of claim 1, further comprising reacting proteins of the mixture with a reagent for protecting amine groups prior to contacting the mixture with the resin.

9. (Original) The method of claim 1, wherein the contacting is done under aqueous conditions in the presence of a source of hydroxide ion, said conditions resulting in β -elimination of the glycosyl group from the glycosylated protein to result in an unsaturated intermediate, said conditions sufficient to result in reaction of the nucleophile with the unsaturated intermediate to yield the deglycosylated protein having a deglycosylation site, the deglycosylated protein bound to the solid support via the deglycosylation site.

10. (Original) the method of claim 1, wherein the resin comprises an amino acid residue bound to the solid support, wherein the amino acid residue has a primary or secondary amine group, wherein the primary or secondary amine group is the nucleophile.

11. (Original) The method of claim 10, wherein the amino acid residue is isotope labeled, and wherein the isotope labeled amino acid residue remains bound to the deglycosylated protein when the deglycosylated protein is released from the solid support.

12. (Original) The method of claim 1, wherein the resin comprises an amino acid residue bound to the solid support, wherein the amino acid residue has a thiol group, wherein the thiol is the nucleophile.

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13. (Original) The method of claim 12, wherein the amino acid residue is isotope labeled, and wherein the isotope labeled amino acid residue remains bound to the deglycosylated protein when the deglycosylated protein is released from the solid support.

14. (Original) The method of claim 1, wherein the resin comprises a peptide bound to the solid support, wherein the peptide has a primary or secondary amine group, wherein the primary or secondary amine group is the nucleophile.

15. (Original) The method of claim 14, wherein the peptide is isotope labeled, and wherein the isotope labeled peptide remains bound to the deglycosylated protein when the deglycosylated protein is released from the solid support.

16. (Original) the method of claim 1, wherein the linker comprises a tag, and wherein the tag remains bound to the deglycosylated protein when the deglycosylated protein is released from the solid support.

17. (Original) The method of claim 16, wherein the tag is selected from a mass tag, a fluorescent tag, an affinity tag, or a chemical group having a specific reactivity.

18. (Original) The method of claim 1, wherein the linker comprises a cleavable group which is stable under the conditions under which the resin is contacted with the mixture of glycosylated proteins and unglycosylated proteins, but which is labile under the conditions used for release of the deglycosylated protein from the solid support.

19. (Original) The method of claim 1, wherein the deglycosylated protein is released from the solid support by exposing the resin bound deglycosylated protein to light.

20. (Original) The method of claim 1, wherein the deglycosylated protein is released from the solid support by exposing the resin bound deglycosylated protein to acid.

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21. (Original) The method of claim 1, wherein the deglycosylated protein is released from the solid support by exposing the resin bound deglycosylated protein to a hydride.

22. (Original) The method of claim 1, wherein the deglycosylated protein is released from the solid support by exposing the resin bound deglycosylated protein to an organometallic reagent.

23. (Original) The method of claim 1, wherein the deglycosylated protein is released from the solid support by exposing the resin bound deglycosylated protein to oxidative reagents.

24. (Withdrawn) A kit comprising a resin that comprises a nucleophile bound to a solid support via a linker, wherein the nucleophile is capable of reacting with a glycosylated protein under conditions conducive to β -elimination of the glycosyl group to result in deglycosylation of the glycosylated protein and concomitant formation of a covalent bond bonding the deglycosylated protein to the solid support; the resin included in a package,

25. (Withdrawn) The kit according to claim 24 wherein the package further includes a reagent selected from a solution effective to release the deglycosylated protein from the resin, reagents for dephosphorylating the mixture of proteins, reagents for digesting the mixture of proteins, reagent for protecting amine groups.

26. (Withdrawn) The kit according to claim 24 wherein the linker comprises a cleavable group and a tag; wherein the tag is bound to the nucleophile, and wherein the cleavable group can be cleaved to release the deglycosylated protein from the solid support under conditions wherein the tag remains bound to the deglycosylated protein.